

REACTIONS IN SUPERCRITICAL CARBON DIOXIDE EFFICIENT PRODUCT FRACTIONATION FOLLOWING ENZYMATIC AROMA SYNTHESIS

T.Gamse^{*}, G.Kracker-Semler, R.Marr

Institute of Thermal Process and Environmental Engineering

Graz University of Technology

Inffeldgasse 25/C, A-8010 Graz, Austria

Email: Thomas.Gamse@TUGraz.at Fax: ++43 316 873 7472

The efficiency of supercritical CO₂ for enzymatic synthesis of short chain geranylestere (geranyl butyrate and geranyl caprylate) is already shown. These aroma substances are used as natural aroma in food stuff industry. Based on these results this paper deals with the fractionated separation of the aroma compounds following the reaction step. A simple change of pressure and temperature of CO₂ in the down-stream phase leads to high concentrated products, so that SC-CO₂ can act as well as reaction as separation solvent for this process.

INTRODUCTION

Enzymes act in nature in aqueous solutions. Many enzymes are associated with more or less nonpolar cellular structures, especially membranes. This led to the conclusion, that only a monolayer of water around the enzyme molecule is truly necessary. From a biotechnological perspective, there are a lot of advantages of conducting enzymatic conversions in monophasic organic solvents as opposed to water [1]: high solubility of most organic (non polar) compounds in nonaqueous media; ability to carry out new reactions impossible in water because of kinetic or thermodynamic restrictions; reduction of water-dependent side reactions; insolubility of enzymes in organic media, which permits their easy recovery and reuse. Using supercritical carbon dioxide (SC-CO₂) instead of organic solvents in biocatalysis presents several additional advantages. For example, gaseous reactants such as oxygen and hydrogen are miscible with supercritical carbon dioxide, making oxidase-catalyzed reactions possible. Very often the performance of biocatalysed processes in organic solvents is low. By using supercritical carbon dioxide an integrated production process can be performed, because SCCO₂ can act as solvent for the reaction and as a separation medium after the reaction. Therefore no change from the reaction medium to the downstream processing (separation) medium is necessary. The overall performance of such an integrated production process can be higher than the multi media process, using conventional organic media [2]. Using organic solvents for enzymatic reactions especially the costs of the separation step can be more than 70% of the total process costs. For this separation purpose SC-CO₂ offers an great advantage of changing solvent power by adjusting only pressure and temperature. The reaction step of a biocatalytic production process using SC-CO₂ as process medium for geranyl butyrate and geranyl caprylate is already published [3 - 5]. In this study the separation

step following the enzymatic reaction has been investigated. Synthetically mixtures of geranyl butyrate and 2-propanol, of same concentrations as produced in the batch reactions, were solved in supercritical CO₂. This supercritical mixture was separated in 2 following separators, operated at different pressure und temperature conditions. Beside optimisation of operation conditions of the separators the influence of different compositions of initial mixture were tested on the separation efficiency and the purity of the products.

1 - MATERIALS AND METHODS

1.1 Materials

geranyl butyrate, 98.3% and 2-propanol, > 99% (Sigma Aldrich, Germany); carbon dioxide with purity >99.94 % (v/v) and a dewing point lower than - 60°C (Linde, Austria). All chemicals were used without further purification.

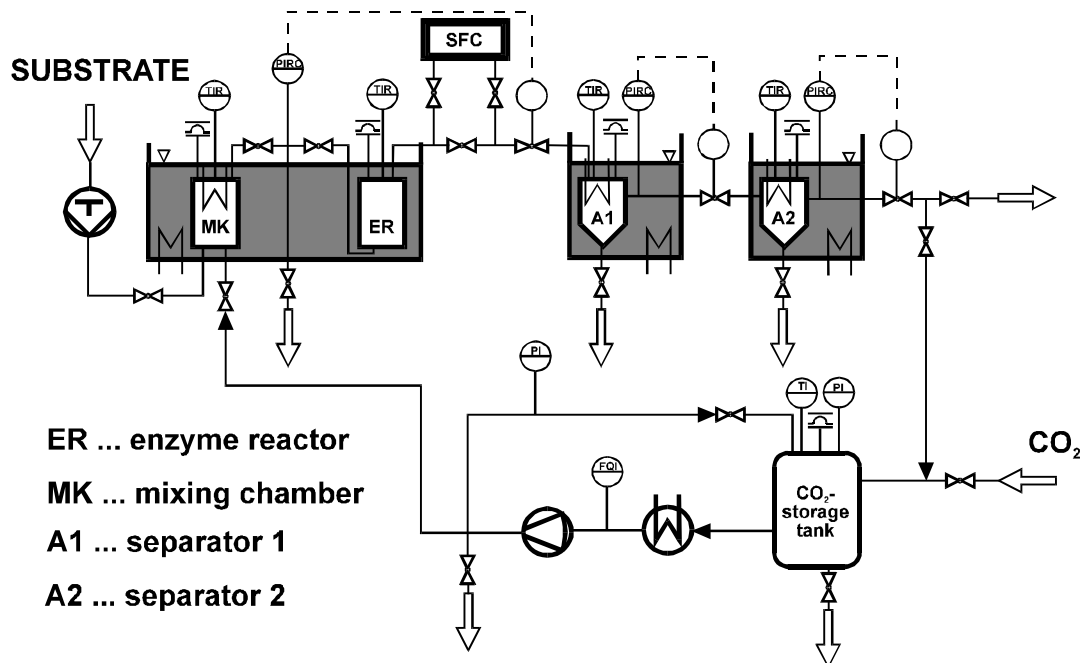


Figure 1: Experimental set-up for fractionation experiments

1.2. Set-up for product fractionation

For the product fractionation tests the continuous operating plant for enzymatic reactions was adapted, which is available at the institute and shown in Figure 1. The enzyme reactor was not in operation for these tests, because a mixture of products was introduced into the mixing chamber ($p_{\max} = 400$ bar, $T_{\max} = 100^{\circ}\text{C}$, $V = 150$ ml, sight glasses over total reactor height on both sides) and solved in supercritical CO₂. For better mixing effect a magnetic stirrer is placed into the mixing chamber and by the sight glasses it can be observed that there is really one supercritical phase without any droplets of liquids. This mixture passes over a pressure regulated valve into the first and by another one into the second separator (each separator: $p_{\max} = 250$ bar, $T_{\max} = 100^{\circ}\text{C}$, $V = 150$ ml, including inside heat exchanger tube with same temperature as water bath). All pressure regulating valves are heated by heating belts to prevent a temperature change because of the Joule-Thomson effect. The CO₂ leaving the

second separator can be recycled by condensing and cycling into the storage tank. In all these experiments the CO₂ was expanded to the atmosphere after passing a gas flow meter for measuring the CO₂ flow rate. The mixing chamber and the two separators are placed in separated water baths, so that for each vessel temperature can be adjusted independent. Pressures and temperatures of all vessels and of entering CO₂ are measured and additionally the temperatures of all water baths, each thermostated by temperature controlled heat exchangers. The flow rates of as well the entering liquid mixture as of the outgoing CO₂ are measured volumetrically.

1.3. Analysis

The concentration of 2-propanol in the propanol - geranyl butyrate mixture was analysed in a Paar DMA 45 densimeter. For calibration as well the two pure compounds as mixtures of different composition were analysed. Based on this calibration curve for all samples from the separation experiments the composition was determined by measuring the density. To remove residual CO₂ from the samples they were placed in an ultrasonic water bath before analysing.

2 - RESULTS

The binary phase equilibria of the different substances in supercritical CO₂ were already measured, calculated and published [6]. Based in these results the product separation was performed.

2.1. Optimisation of separation pressures and temperatures

The parameters for the mixing chamber were 150 bar and 40°C, because under these conditions the enzymatic reactions in the batch apparatus were performed. The initial mixture contains 20 wt% geranyl butyrate and 80 wt% 2-propanol, which is the composition after the enzymatic reaction.

For the first series the flow rate of the liquid mixture was 60 ml/h and 1,5 kg/h of CO₂. The experiments lasted for 2 hours taking samples from both separators every 30 minutes. The second separator was operated at 40 bar and 40°C and only the parameters of the first separator were varied, temperature between 40°C to 80°C and pressure between 70 to 100 bar. Figure 2 shows the results when operating the first separator at 80 bar and different temperature levels. It is obvious that an increase in temperature of the first separator increases the concentration of geranyl butyrate in the first fraction from 40.9 wt% at 40°C to 81.7 wt% at 80°C. A higher temperature lowers also the geranyl butyrate content in the second separator, where the main part of 2-propanol is separated.

The influence of different separation pressure in step 1 at a constant temperature of 80°C is shown in Figure 3. A pressure increase up to 90 bar results in higher geranyl butyrate concentration in the first separator, while at 100 bar separation gets worse. The geranyl butyrate concentration of the second fraction increases continuously with increasing pressure of the first separator.

For producing high concentrations of the aroma compound geranyl butyrate best separation conditions are 90 bar and 80°C for separator 1 and 40 bar and 40°C for separator 2.

In a further series the temperature of the second separator was changed from 40°C to 60°C but this temperature change has a negative influence on the concentration of geranyl butyrate in both separators.

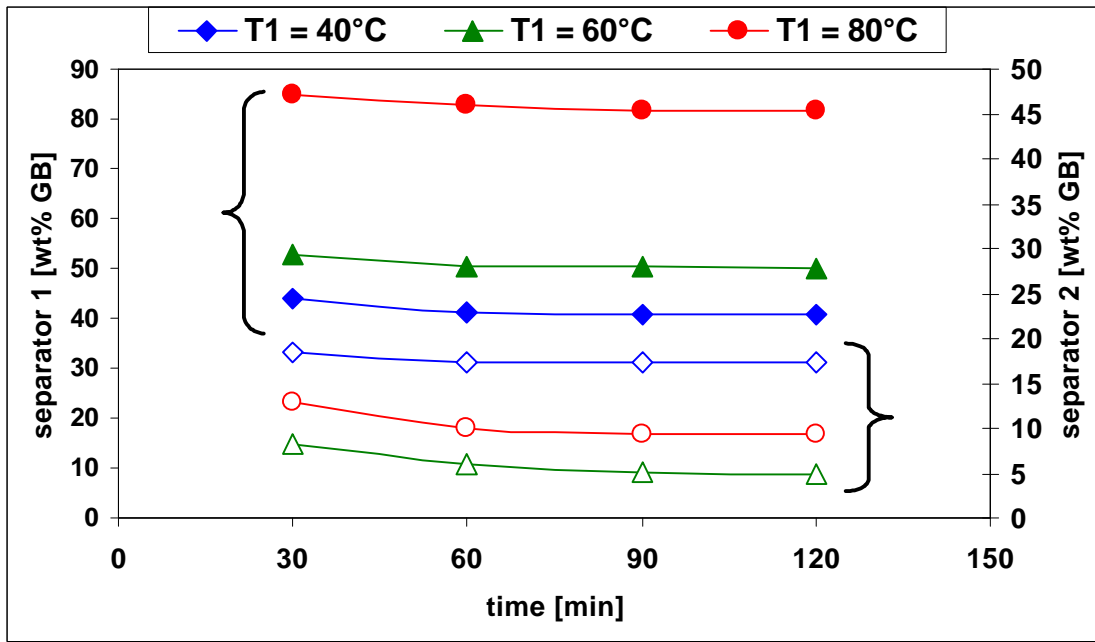


Figure 2: Influence of temperature of the first separator on the geranyl butyrate concentration in both fractions, $p_{\text{sep } 1} = 80 \text{ bar}$, $p_{\text{sep } 2} = 40 \text{ bar}$, $T_{\text{sep } 2} = 40^\circ\text{C}$, full symbols = concentration in separator 1, open symbols = concentration in separator 2

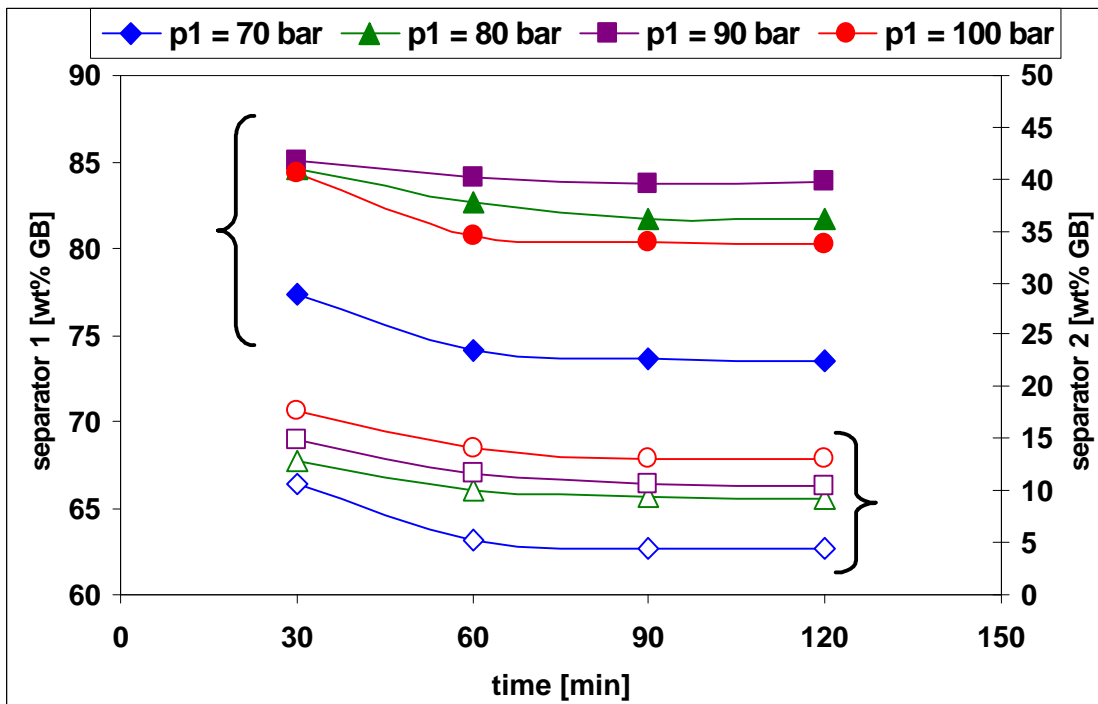


Figure 3: Influence of pressure of the first separator on the geranyl butyrate concentration in both fractions, $T_{\text{sep } 1} = 80^\circ\text{C}$, $p_{\text{sep } 2} = 40 \text{ bar}$, $T_{\text{sep } 2} = 40^\circ\text{C}$, full symbols = concentration in separator 1, open symbols = concentration in separator 2

2.2 Influence of different geranyl butyrate concentration

The concentration of geranyl butyrate in the initial mixture was varied from 10 wt % up to 30 wt%. As given in Figure 4 for both separators there is no difference in the separation effect between 20 wt% and 30 wt% geranyl butyrate in the initial mixture. This is very positive because if conversion rates during the enzymatic reactions will increase the same high concentrations of geranyl butyrate can be produced without changing any operation parameters.

Surprising is the low concentration of geranyl butyrate in the first separator if only 10 wt% geranyl butyrate is present in the raw material. An explanation might be that 2-propanol saturates at the given conditions in separator 1 up to a certain level with geranyl butyrate, so that at low geranyl butyrate concentrations most of the geranyl butyrate is withdrawn to the second separator by the 2-propanol.

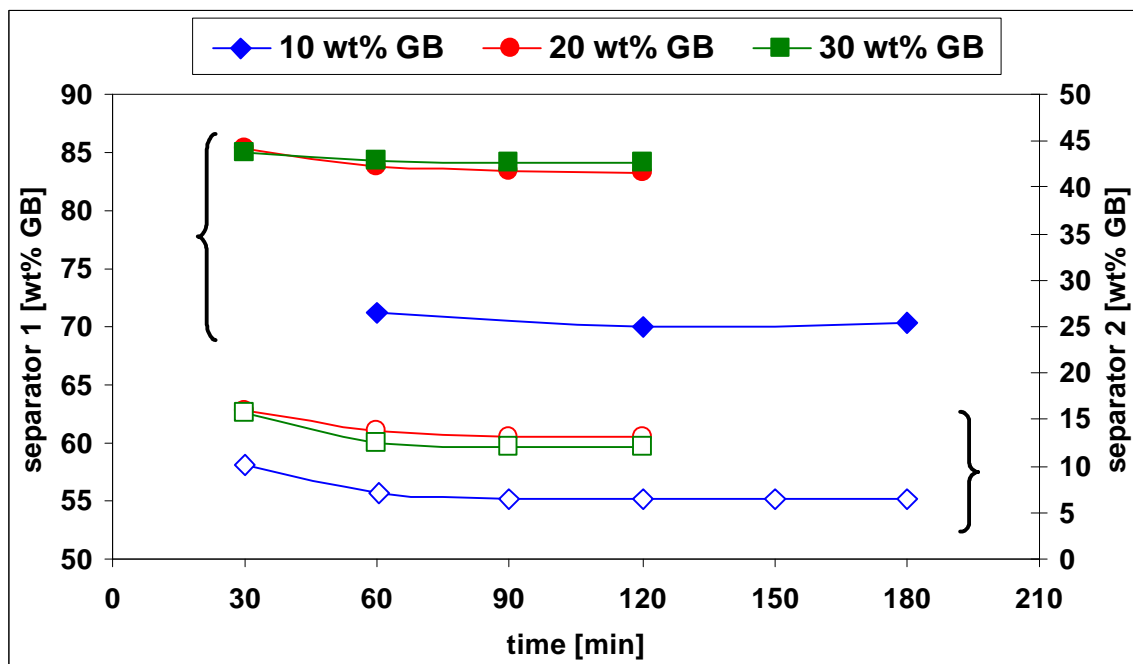


Figure 4: Influence of initial geranyl butyrate concentration on both fractions, $p_{\text{sep } 1} = 90$ bar, $T_{\text{sep } 1} = 80^{\circ}\text{C}$, $p_{\text{sep } 2} = 40$ bar, $T_{\text{sep } 2} = 40^{\circ}\text{C}$, full symbols = concentration in separator 1, open symbols = concentration in separator 2

CONCLUSION

Using supercritical CO_2 for enzymatic aroma synthesis results in a simple and efficient separation of the products in the down-stream phase. As shown concentrations of geranyl butyrate up to 85 wt% can be achieved in the first separator by simple pressure and temperature change of the supercritical fluid. This means that this product fraction is over 4 times more concentrated than the initial mixture coming from the enzymatic reaction. The 2-propanol fraction of the second separator contains still 5 to 15 wt% geranyl butyrate, which will be loss of product if not recovered by any further process.

Especially this simple separation step shows the advantages of supercritical fluids over organic solvents. The reaction rates are comparable to these ones achieved in hexane, but for the product separation supercritical CO₂ gives the great advantages, because no further separation step is necessary. The products are completely free from any solvent, which is in the case of organic solvents a very difficult to handle and cost intensive separation step. Further the products are high concentrated and depending on the field of usage no further concentration step might be necessary.

The use of supercritical fluids will be tested in future for further product mixtures obtained as well by enzymatic reactions in supercritical fluids as in organic solvents. Sometimes it makes no sense to perform the enzymatic reaction in supercritical CO₂ because of a lot of parameters to be optimised. But for a simple and effective separation of the obtained reaction mixture supercritical fluids offer a promising method to produce high concentrated products of high quality.

REFERENCES :

- [1] DORDICK, J.S., *Enzyme Microb. Technol.*, 11, **1989**, p.194.
- [2] MARTY A., COMBES D., CONDORET J.S., *Biotechnol. Bioeng.*, 43, **1994**, p.497.
- [3] BAUER, C., GAMSE, T., MARR, R., *Proc. of 15th Int. Congress of Chemical and Process Engineering, CHISA 2002*, 25.-29.8.2002, Praha, cdrom
- [4] BAUER, C., GAMSE, T., MARR, R., *Proc. of 4th Int. Symp. High Pressure Technology and Chemical Engineering*, 22.-25.9.2002, Venice, p.915
- [5] BAUER, C., GAMSE, T., MARR, R., *Bioprocess and Biomaterial Engineering*, paper submitted
- [6] GAMSE, T., DEPOLI, G., MARR, R., *Proc. of 4th Int. Symp. High Pressure Technology and Chemical Engineering*, 22.-25.9.2002, Venice, p.279

The work was supported by a grant of the Austrian Science Fund FWF (proj. P15291).